IJARST

International Journal of Advanced Research in Science and Technology

journal homepage: www.ijarst.com

ISSN 2319 – 1783 (Print)

ISSN 2320 – 1126 (Online)

Hybanthus enneuspermus (L) F. Muell. leaf broth mediated synthesis of silver nanoparticles

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ARTICLE INFO

Article history:

Received	10 Oct. 2014
Accepted	11 Nov.2014
Available online	17 Nov.2014

Keywords:

H. enneuspermus,
Silver nanoparticles,
Reaction medium,
SEM, TEM.

ABSTRACT

The present study is aimed to investigate the synthesis of silver nanoparticles using leaf broth of *Hybanthus enneuspermus* (Family: Violaceae). The freshly prepared leaf broth of *H. enneuspermus* was pale yellow in colour and turned to brown within thirty minutes and became dark brown in colour within twenty four hours when exposed to 1mM aqueous silver nitrate. This indicates the synthesis of silver nanoparticles from silver nitrate through bio-reduction. The synthesized silver nanoparticles were characterized by the UV-Visible spectroscopy (UV-Vis), Fourier Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD) analysis, Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray (EDX) spectroscopy, Transmission Electron Microscopy (TEM) analysis. TEM elucidates the size and shape of the synthesized silver nanoparticles. The size varies between 05 and 30nm with the mean 15±3.38nm. As a green approach and cost effective nature, the leaf broth of *H. enneuspermus* mediated synthesis of silver nanoparticles becomes an alternative to physical and chemical methods. Further this synthesis protocol becomes an eco-friendly route as it is not involved of any toxic chemicals.

Introduction:

An eco-friendly synthesis of silver nanoparticles using higher plants is an important fascinating aspect in the field of Nanotechnology [1-4]. The silver nanoparticles can be synthesized using plant, either intracellular or extracellular [5]. The use of plant extracts is non-toxic to humans without any side effects and most effective against bacteria, viruses and other eukaryotic microorganisms [6].There are different kinds of metal nanoparticles synthesized using plant extracts such as Ag, Pt, Au and Pd [7-10]. Among them, silver nanoparticles are proved to be the most effective as they have good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms [11]. In the recent past, several reports are available to explain the biological synthesis of silver nanoparticles. [12-14]. The use of plant resources for the synthesis of silver nanoparticles have many advantages over traditional chemical and physical approaches [15].This is cost effective, capable of large scale production, easy to follow and not involved of toxic chemicals [16-23,1,3]. In that line, the present study is aimed to evaluate the reducing ability of leaves of *H. enneuspermus* to reduce silver nitrate and synthesize silver nanoparticles.

Material and methods:

The silver nitrate ($AgNO_3$, 99.9%) used in the present study is obtained from Himedia Laboratories Pvt Ltd (Mumbai, India). The plant *H. enneuspermus* (Figure 1) belongs to Violaceae. The fresh and healthy leaves of *H. enneuspermus* were collected from the Botanical Garden of Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India. The plant has gained much attention among ethnobotanists due to its antimicrobial and antiplasmodial action [24,25], anti gonorrhoeac, anti-inflammatory, anti tussives, anticonvulsant and freeradicals scavenging activity, and also used in the treatment of jaundice and aqueous extract possessed hypoglycaemic activity [26,27]. Traditionally this plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhoea, leucorrhoea, dysuria, sterility, diabetes and bowel complaints [28-30].

Figure: 1. *Hybanthus enneuspermus*

The collected leaf sample was thoroughly washed with tap water followed by double distilled water to remove the surface contaminants and dried for 48 hours under shade. The dried leaves were taken and ground to make fine powder using mixer and sieved using 20 mesh sieves to get uniform size range. 10g of sieved powder was added to 100ml of distilled water and boiled at 70˚C for ten minutes to prepare the leaf broth. 10ml of freshly prepared leaf broth was re-suspended in 190 ml of 1mM aqueous solution of silver nitrate and this mixture was used as reaction medium [31,32]. The reaction medium was kept in an Incubator cum shaker (Orbitek-Model) with 250 rpm at 20º C for 24 hours.

Characterization of silver nanoparticles:

The characterization of synthesized silver nanoparticles was performed through the following analyses: UV-Visible spectroscopy (UV-Vis) (Labomed Model UV- D3200), Fourier Transform Infrared spectroscopy (FTIR) (Shimadzu), X-ray Diffraction (XRD) analysis (Shimadzu, XRD 6000), Scanning Electron Microscopy (SEM) (Hitachi S-4500) coupled with Energy Dispersive X-Ray (EDX) spectroscopy and Transmission Electron Microscopy (TEM) (Philips-Techno 10 instrument operated at an acceleration voltage of 200KV with resolution of 0.3nm).

Result and Discussion:

UV-Visible Spectrum of Silver Nanoparticles:

The aqueous leaf broth of *H. enneuspermus* was pale yellow in colour, and after the addition of aqueous silver nitrate, the colour changed from pale yellow to brown within 30 minutes and became dark brown within 24 hours of reaction time (Fig 2 Inset). It indicates the formation of silver nanoparticles. Ankanna *et al*., (2010) [33] also reported that the colour change is due to the coherent excitation of all the free electrons within the conduction band, leading to an in-phase oscillation which is known as Surface Plasmon Resonance (SPR). In the present study the synthesized silver nanoparticles exhibited dark brown colour in aqueous reaction medium. Interestingly, the leaf extract of *Stemodia viscosa* and fungal extract of *Verticillium fungicola* made the colour change within few minutes and twenty four hours respectively [34]. The leaf extract of *Epipremnum aureum* reduced the silver nitrate and made the colour change from colourless to brownish yellow within two hours [35]. The time taken for the colour change in the reaction medium varies from plant to plant by which the reduction is possible. This may be ascribed to the quantitative variation in the formation of silver nanoparticles or availability of H^+ ions to reduce the silver [36]. The UV-Vis analysis from 330 to 540 nm revealed that maximum absorbance peak (λ max) was at 430 nm and the absorbance raised up to 0.37 a.u. at 24h incubation period (Fig 2). However, the λ max in SPR band was found to be at 430nm due to the silver nanoparticles synthesized using *Parthenium hysterophorus* [37]; 430 nm using *Morinda pubescens* [38]; 405nm and 480nm using *Enicostema hysopifolium* and *Rauvolfia tetraphylla* respectively [39]; 430 nm using *Magnolia kobus, Pinus desiflora, Platanus orientalis* [15] and *Tecoma stans* [40].

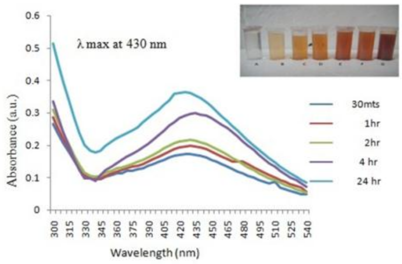

Figure: 2. UV –Visible absorption spectra of silver nanoparticles synthesized by leaf broth of *H. enneuspermus.* The inset shows the colour change of the reaction medium (left to right) A-Control (Aqueous silver nitrate); B-0 hr, C-30 minutes, D-1 hr, E-2 hr, F-4 hr and G-24 hr respectively.

FTIR Spectroscopic Analysis:

FTIR measurement was carried out to identify the possible biomolecules present in the leaf broth of *H. enneuspermus* shown in Figure 3. The FTIR spectral analysis observed in the region of 2000-1000 cm^{-1} are 1120.56, 1191.93, 1336.58, 1400.22, 1454.23 and 1666.38cm^{-1}. The broad absorption band at 1120 cm^{-1} indicates the presence of alcoholic groups [41]. The absorption band at 1400 cm^{-1} is possibly due to the bending tendency of symmetric CH_3 groups within the acetyl and pyruvyl groups as substituents [42]. The absorption band at 1191cm^{-1} corresponding to polysaccharides [43,44]. The band at 1666 cm^{-1} is nearer to the characteristic infrared band representing 3_{10}-helics [45].

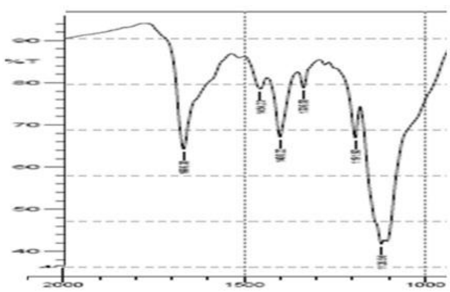

Fig :3. FTIR spectrum of synthesized silver nanoparticles using leaf broth of *H. enneuspermus* with silver nitrate

XRD Analysis:

Figure 4 shows the X-ray diffraction pattern obtained for the synthesized silver nanoparticles using leaf broth of *H. enneuspermus*. The observed XRD pattern had four intense peaks of 2 θ values corresponding to 38^0(111), 46^0(200), and 65^0 (220) and 78^0(311). The XRD pattern revealed that the silver nanoparticles formed are face centered cubic (fcc) structures of silver. The observed peaks suggest that there is crystallization of bio-organic phase on the surface of the silver nanoparticles [46,47]. The size of the crystalline nature of the silver nanoparticles was calculated using Scherrer's formula as $D= 0.97\lambda/\beta \cos \theta$, where D is particle size, λ is wavelength of x-ray, β is full width at half maxima for a peak, and θ is the corresponding Bragg angle [48,49]. We found that the average size of the silver nanoparticles was 15 nm.

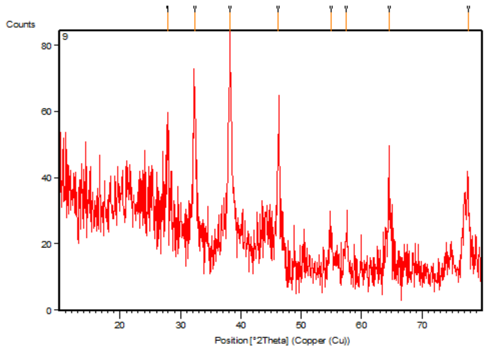

Figure: 4. XRD pattern of silver nanoparticles synthesized using leaf broth of *H. enneuspermus*

SEM and EDX Analyses:

The surface morphology (size and shape) of the silver nanoparticles is shown in Fig 5. The SEM image of silver nanoparticles reveals that they are spherical shaped with the size ranges from 20-50 nm (~). However, the spherical shaped silver nanoparticles with a diameter ranged from 20 to 50 nm were synthesized using *Enicostema hysopifolium* and *Rawvolfia tetraphylla* [38]. The size of silver nanoparticles is ranging from 5 to 50 nm using *Cardiospermum helicacabum* [50]; 20-40nm using *Lepisanthes tetraphylla* [51]; 30-50nm using *Merremia tridentata* [52] and 5-30 nm using *Odina wodier* [53].

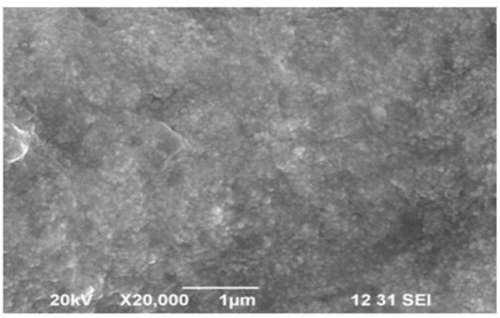

Fig :5. SEM image of silver nanoparticles synthesized using leaf broth of *H. enneuspermus*

Energy Dispersive X-Ray (EDX) analysis revealed the presence of elemental silver signal synthesized using leaf broth of *H. enneuspermus* shown in Fig 6. The EDX peak of Ag along with Ca, O and Cl as the mixed components present in the reaction medium. A weak signal of oxygen is recorded and it may possibly due to the presence of enzymes or proteins present in the reaction medium [54].

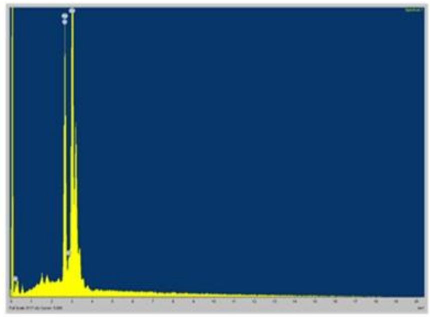

Fig :6. EDX image of silver nanoparticles synthesized using leaf broth of *H. enneuspermus*

TEM Analysis:

Figure 7a shows a high resolution TEM image of synthesized silver nanoparticles using *H. enneuspermus*. The silver nanoparticles obtained are seen as polydispersed and almost spherical in shape with size ranging from 5-50 nm (~) with the mean 15±3.38nm (Fig 7b). There are reports in the synthesis of different sized and shaped silver nanoparticles. For example, silver nanoparticles with 33.6 nm obtained using *Allium cepa*. [19]. Zargar et al., (2011) [55] synthesized silver nanoparticles with an average size of 18.2 nm using *Vitex negundo*. The spherical and cubic shaped silver nanoparticles with the size from 32-53 nm were obtained using *Phyllanthus amarus* [40].

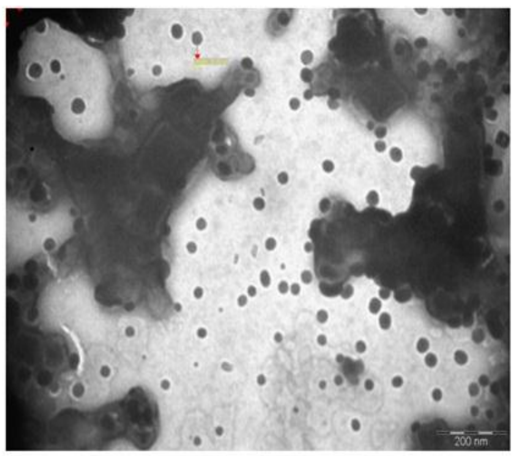

Fig :7a. TEM image of silver nanoparticles synthesized using leaf broth of *H. enneuspermus*

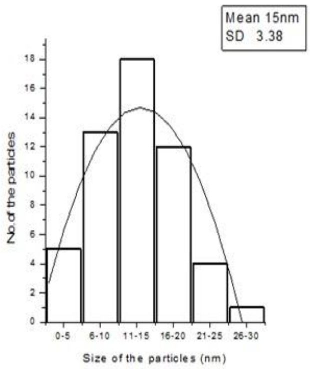

Fig :7b. Size distribution of silver nanoparticles synthesized using the leaf broth of *H. enneuspermus* measured by TEM analysis

Conclusion:

This is a simple, rapid, cost effective and eco-friendly approach for the synthesis of silver nanoparticles at room temperature using leaf broth of *H. enneuspermus*. The biomolecules present in the leaf broth act as reducing and stabilizing agents. Interestingly, it is believed that the polysaccharides, alcoholic, acetyl and pyruvyl groups present in the leaf broth of the *H. enneuspermus* may act as the reducing agent which reduces the silver ions to silver nanoparticles. XRD patterns obtained the silver nanoparticles are Face Centered Cubic structure of silver with average size is 15 nm. The EDX analysis shows a strong elemental silver with weight of sixty four percent. The silver nanoparticles showed polydispersity and particles size ranged from 05 to 50 nm with the mean 15±3.38nm.

Acknowledgement:

The financial support by Science and Engineering Research Board, Department of Science and Technology, New Delhi to carry out this research project is greatly acknowledged by the authors. Authors also thank the Principal and Management of Ayya Nadar Janaki Ammal College, Sivakasi for providing necessary facilities.

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